Clinical Pharmacogenomics: Why We Don't Have It. How We Can Get It.

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ACCP Meeting
Bethesda, September 11th
MD 2005

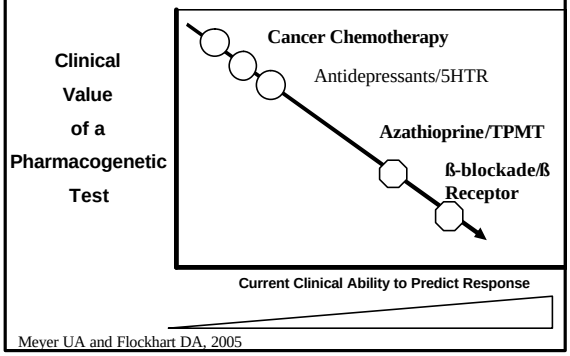
Purposes of Pharmacogenomics

To elucidate mechanism

To predict response

Pharmacogenetic Principle 1:

Value Decreases when Current Predictive Ability is High



Meyer UA and Flockhart DA, 2005

Pharmacogenetic Principle 1:

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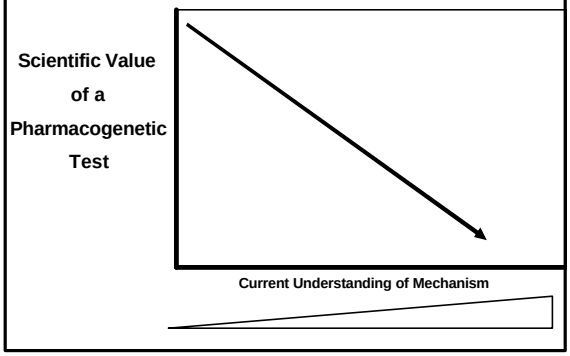


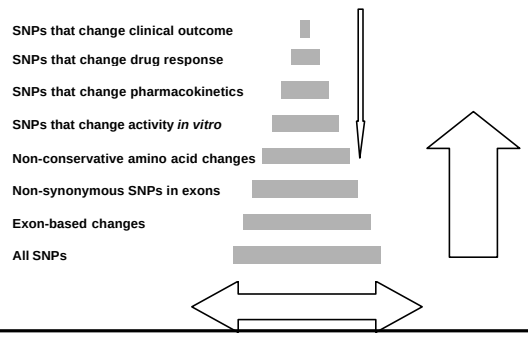
Why we Don't Have it

- Prediction of Dose "Challenging"
- Flawed Poster Children
- Few Prospective Trials of Genetically-Driven Clinical Practice vs Standard of Care
- Little Incremental Value Apparent in a Quality Driven Clinical Environment

Progress in the Last Ten Years?

- FDA no longer a barrier
- Price no longer a barrier
- Utility in the Pharmaceutical Industry widespread
- Warfarin

Hierarchy of Pharmacogenetic Information



How to Get Clinical Pharmacogenomics?

1. Choose a Disease that Matters to a LOT of People
2. Choose a disease where the effects of therapy are hard to predict using currently available technology in the clinic.

i.e. NOT an antihypertensive, or antihistamine or analgesic

Breast Cancer

- 65, 000 women in North America will be diagnosed on 2005
- 2nd most common malignancy in women
- Lifetime risk for breast cancer among women is 1 in 7.

Treatment of Breast Cancer has been "Personalised" from the start.

- Prevention: Tamoxifen
- Surgery
- Chemotherapy:
  - Anthracyclines
  - Taxol
  - Cyclophosphamide/Methotrexate/5-FU
- Endocrine therapy: ER-dependant
  - Aromatase Inhibitors
  - SERMs
- Herceptin – HER2 neu-dependant

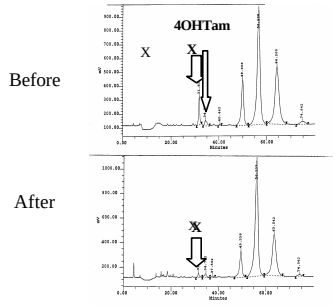
Tamoxifen

- The first SERM
- 70% of breast cancers are ER+, until 2000 all were treated with tamoxifen
- The only approved preventive therapy for breast cancer
- 1 million lives are saved per year by tamoxifen

Case Report

- 45 year-old woman presented with intense, intolerable hot flashes after being prescribed 20 mg of tamoxifen per day for a week.
- Placed on 10 mg per day of paroxetine for depression
- Resolution of hot flashes within a week
- Hot flashes resumed when taken off paroxetine

Paroxetine Administration Decreased the Concentration of One Metabolite



Lee, Jones, Desta, Flockhart: J. Chromat B, 2001; 791:245-53.

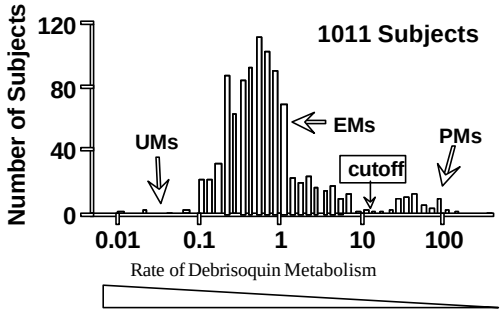
DRUGS METABOLIZED BY KNOWN P450'S
Indiana University Medical Center

1A2	2C19	2C9	2D6	2E1	3A
Clozapine	Amitriptyline	Diclofenac	Amitriptyline	Acetaminophen	Alprazolam
Cyclobenzaprine	Clomipramine	Flurbiprofen	Clomipramine	Chlorzoxazone	Astemizole
Fluvoxamine	Cyclophosphamide	Ibuprofen	Codeine	(Parafon Forte®)	Buspirone
Imipramine	Diazepam	Losartan	Desipramine	Ethanol	Calcium Channel Blockers
Mexiletine	Imipramine	(NOT candesartan)	Dextromethorphan	Enflurane	Carbamazepine
Propranolol	Lansoprazole	Naproxen	Imipramine	Halothane	Cisapride
Theophylline	Nelfinavir	Phenytoin	Metoprolol	Isoflurane	Cyclosporine
	Omeprazole	Piroxicam	Nortriptyline		HIV Protease Inhibitors
	Phenytoin	Sulfamethoxazole	Oxycodone		Lovastatin
		Tolbutamide	Paroxetine		NOT pravastatin
		Warfarin	Propranolol		Simvastatin
			Risperidone		Midazolam
			Thioridazine		Tacrolimus
			Timolol		Triazolam
		INHIBITORS			
Cimetidine	Cimetidine	Amiodarone	Amiodarone	Disulfiram	Amiodarone
Ciprofloxacin	Fluoxetine	Fluconazole	Fluoxetine		Cimetidine
Fluvoxamine	Fluvoxamine	Fluoxetine	Haloperidol		Clarithromycin
Ofloxacin	Ketoconazole	Isoniazid	Indinavir		Erythromycin
Ticlopidine	Lansoprazole	Paroxetine	Paroxetine		NOT Azithromycin
	Omeprazole	Ticlopidine	Quinidine		Grape Fruit Juice
	Paroxetine	Zafirlukast	Ritonavir		Itraconazole
	Ticlonidine		Sertraline		Ketoconazole
		INDUCERS			
Tobacco	Carbamazepine	Phenobarbital		Chronic Ethanol	Carbamazepine
	Norethindrone	Rifampin		Isoniazid	Glucocorticoids
	NOT phenobarb				Phenytoin
					Rifampin
					Ritonavir
	Absent in 15-30 % of Asians	Absent in ~ 1 % of Caucasians	Absent in 7 % of Caucasians	Hyperactive in ~ 1 % Caucasians	

Paroxetine is a potent and specific inhibitor of CYP2D6 (fluoxetine, venflaxine, quinidine also)

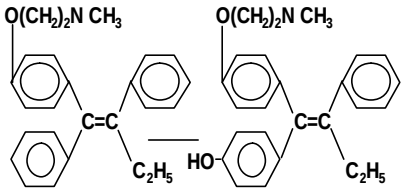
Hypothesis : Hot flashes are being reduced by a paroxetine-mediated drug interaction involving CYP2D6 that reduces active tamoxifen metabolite concentrations.

CYP2D6 Pharmacogenetics

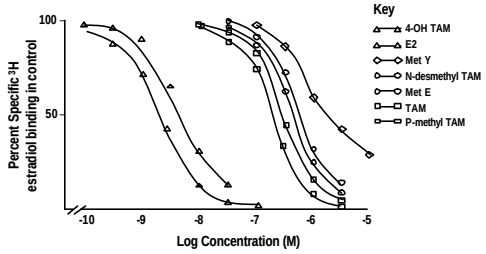


Primary Routes of Tamoxifen Metabolism

4-hydroxylation to active metabolite



Estrogen Receptor Binding of Tamoxifen and its Metabolites



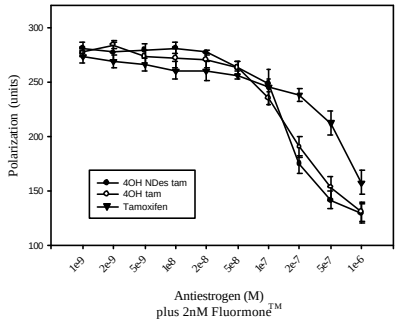
Furr B.J.A., *Pharmac Ther.*, Vol.25, p138, 1984.

Separated, purified, identified and synthesized metabolite X.

= 4-hydroxy-N-desmethyl tamoxifen
= Endoxifen

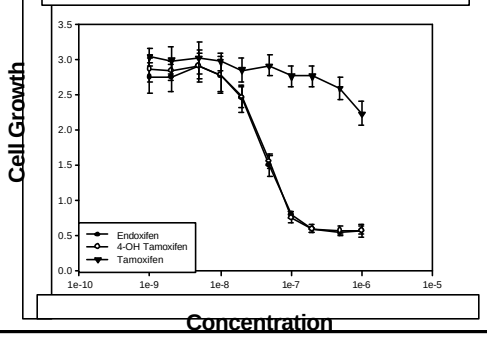
Identified CYP2D6 as the primary catalyst of its formation *in vitro*.

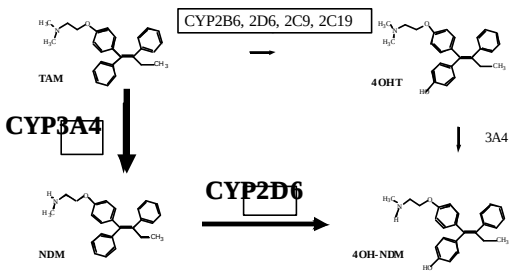
4-OH-Tamoxifen and 4-OH-N-Des-Tamoxifen have equal affinities for Estrogen Receptor a



Johnson MD, Flockhart DA. Breast Cancer Research and Treatment. 2004. *In Press*.

Endoxifen and 4-OH-Tamoxifen are Equipotent as Inhibitors of Estrogen Stimulated Cell Proliferation

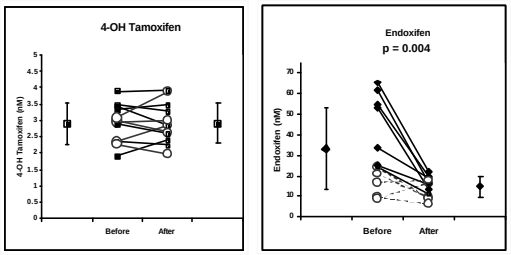




Effect of paroxetine and CYP2D6 genotype on steady state tamoxifen pharmacokinetics

- Enrolled 12 women who experienced hot flashes with tamoxifen and requested treatment.
- Determined CYP2D6 genotype by Affymetrix chip for 19 alleles and by RFLP for 12 variant alleles.
- Open, randomized, fixed interval trial design.
- Tamoxifen 20 mg po qd with then without paroxetine 10 mg po qd.

Paroxetine and CYP2D6 genotype change the plasma concentrations of endoxifen



Flockhart *et al*. JNCI In Press, December 2003

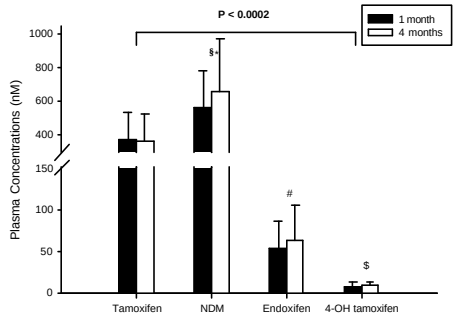
Prospective Pharmacogenetic Trial of the Efficacy and Adverse Effects of Tamoxifen

- Genotypically nested study of 300 women taking tamoxifen measuring tamoxifen and metabolite concentrations:
  - Dynamic measures:
    - hot flashes
    - Plasma lipids: cholesterol, TG, LDL, HDL.
    - bone density
    - coagulation parameters: factor V, fibrinogen concentrations, TFPI, TBG
    - CRP,

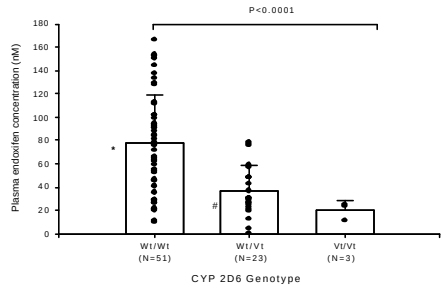
Prospective Tamoxifen Data

Surrogates for Outcome	Pharmacogenetic Tests
• Hot Flashes	• CYP2D6: 8 alleles
• Cholesterol	• ERa:3 alleles
• LDL	• SULT1A1:2 alleles
• HDL	• CYP2B6: 3 alleles
• TBG	• CYP3A5: 2 alleles
• Bone Density	• MDR: 2 alleles
• Coagulation Factors	• eNOS:4 alleles

Endoxifen Concentrations are 5 – 10 fold higher than those of 4-OH-tamoxifen at steady state.



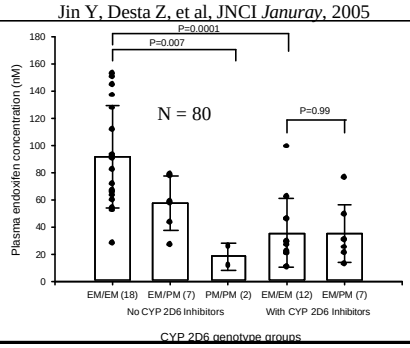
CYP2D6 variant genotype results in lower [endoxifen]



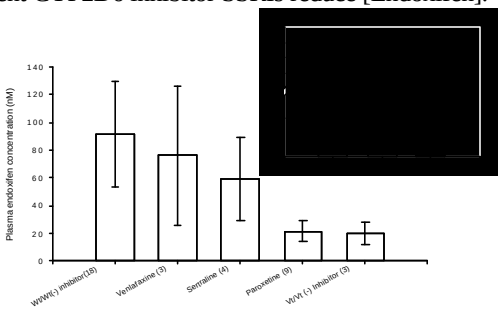
Jin Y, Desta Z, et al, JNCI , 2005.

CYP2D6 variant genotype and CYP2D6 inhibitors lower [Endoxifen]

Jin Y, Desta Z, et al, JNCI *Januray*, 2005



Potent CYP2D6 inhibitor SSRIs reduce [Endoxifen].



Trials to Test the Hypothesis that CYP2D6 Genotype alters Tamoxifen Outcome.

- Prospective trials of tamoxifen vs placebo will not be done again.
- Retrospective trial data are available but no DNA was collected in the 17 trials known to be conducted 1975 - 1991.
- Could paraffin blocks be used?

Genotyping for polymorphic drug metabolizing enzymes from paraffin-embedded and immunohistochemically stained tumor samples.

Pharmacogenetics. 2003 Aug;13(8):501-7.

Rae JM, Cordereo KE, Scheys JO, Lippman ME, Flockhart DA, Johnson MD.

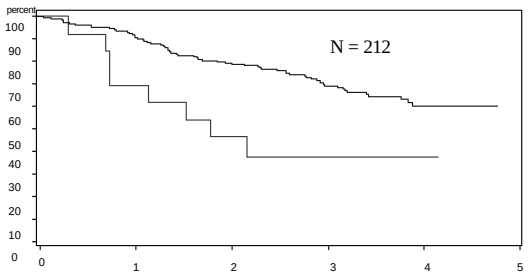
Complete agreement between blood and tumor for polymorphisms in CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A5 and MDR1

Among H & E stained, or immunohistochemically stained samples, only 25/50 amplified and only 14 produced accurate genotypes.

Retrospective Trials with Paraffin Blocks available

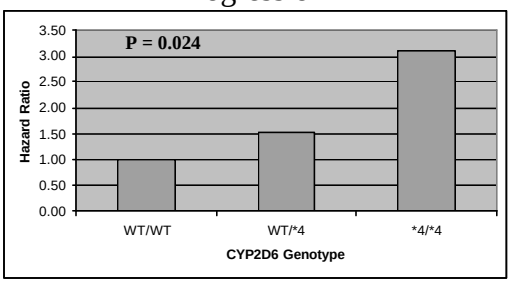
- **89-30-52: Phase III Trial of Adjuvant tam versus tam with fluoxymestrone**
  ~ 1000 patients
- NSABP – P-1 : 11,000 patients

CYP2D6 PM GENOTYPE REDUCED DISEASE-FREE SURVIVAL
IN WOMEN WITH ER+ BREAST CANCER



89-30-52: PHASE III TRIAL OF ADJUVANT THERAPY WITH TAM ALONE OR COMBINED WITH FLU

CYP2D6 Variant Genotype Increased the Risk of Breast Cancer Progression



Adapted from Goetz M, Rae JM *et al*, SABCS, 2004

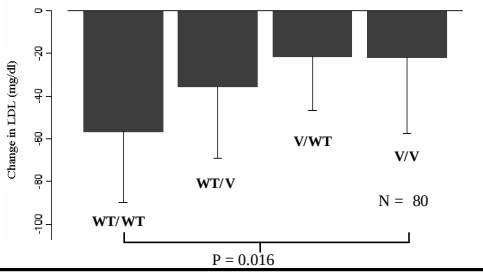
Summary

- Endoxifen is as potent an anti-estrogen as tamoxifen, but is present at 5-10 x greater concentrations
- Endoxifen concentration is sensitive to CYP2D6 genotype
- Potent inhibitors of CYP2D6 reduce endoxifen concentration
- CYP2D6 variant genotype reduced disease free survival in a randomised, prospective trial.

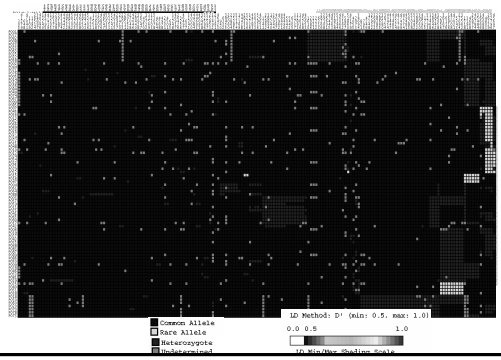
Risks and Benefits of Estrogen Plus Progestin in Healthy Postmenopausal Women :

JAMA 2002;288:321-328.

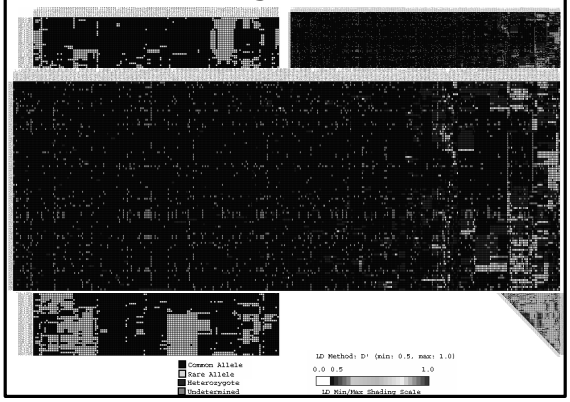
ERa and ERb genotypes alter LDL-cholesterol response to 4 months of Tamoxifen treatment: a Gene-Gene-Drug Interaction



For Genes with a Simple Haplotype structure, tag SNPs may be valuable in Association Studies:CYP2C9



ESR1



Genes-SNPs-Haplotypes

Gene Name	Length (kb)	Exons	Individuals	SNPs >5% / <5%	Haplotypes
ESR1	296	8	90	**136(3)**/177(6)	**168[-]**
CYP2C9	54	9	90	**48(1)**/132(9)	**17[*12*]**

( ) - Exon SNPs ; [ ] – Haplotyes that cover 90% of population

Summary

- A Paucity of Prospective Trials and a Studied Ignorance of the Clinic Represent the Most Important Barriers to Clinical Pharmacogenomics Becoming a Reality
- Multidisciplinary Research Groups involving Genomic Scientists and Experts in Specific Phenotypes are Best Equipped to Make Progress

The Division of Clinical Pharmacology

Indiana University School of Medicine

COBRA: The Consortium on Breast Cancer Pharmacogenomics